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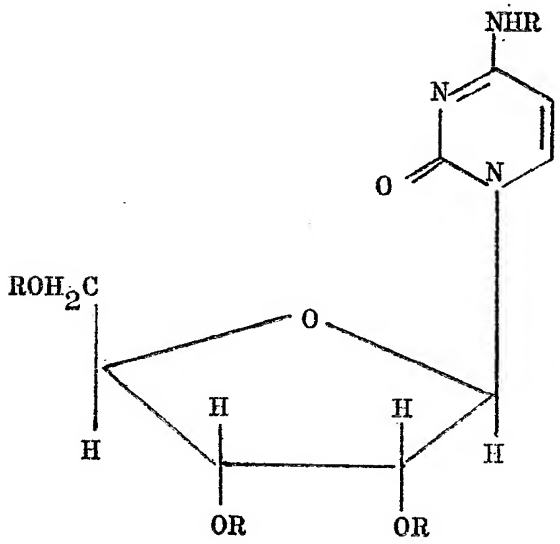
(54) $N^4,O^{2'},O^{3'},O^{5'}$-TETRAACYCLTIDINE

(71) We, TAKEDA YAKUHIN KOGYO KABUSHIKI KAISHA also known as TAKEDA CHEMICAL INDUSTRIES, LTD., a body corporate organised under the laws of Japan, of 27, Doshomachi 2-chome, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to novel $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines and to a process for their preparation.

The invention provides novel $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines of the formula:



(I)

wherein R represents an acyl radical of a fatty acid having from 3 to 18 carbon atoms. We have found that these compounds show excellent pharmacological actions such as remarkable central nervous system activating effects, and that they show excellent results in the treatment of disturbance of consciousness of neuro-psychiatric symptoms e.g. due to head injury, cerebral vascular disturbance or cerebral operation.

The invention also provides a pharmaceutical composition comprising at least one

novel $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines together with a pharmaceutically acceptable carrier or diluent therefor.

The acyl group of the $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines of the formula (I) is an acyl radical of a fatty acid having from 3 to 18 carbon atoms. The acyl group may be derived from any straight chain fatty acid, branched chain fatty acid, saturated fatty acid or unsaturated fatty acid, provided that it has from 3 to 18 carbon atoms. As typical examples of the acyl group, there may be mentioned propionyl, butyryl, isobutyryl, valeryl, isovaleryl, caproyl, octanoyl, lauroyl, palmitoyl, oleoyl, stearoyl and linoleyl radicals.

The $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines (I) may be produced by reacting cytidine with an acid anhydride or an acid halide (e.g. the acid chloride or the acid bromide) of a corresponding fatty acid. Generally, the acid anhydride or acid halide is advantageously employed in an amount in excess of 4 moles, preferably from 5 to 10 moles, relative to cytidine.

Practically, the reaction is carried out in an organic solvent. As the organic solvent, there may be preferably employed pyridine, benzene, chloroform or a mixture thereof. The reaction proceeds smoothly at room temperatures (10°C to 35°C), but the reaction may be conducted with heating or cooling, as conditions demand so as to adjust the reaction velocity.

Examples of the $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines (I) are:

$N^4,O^{2'},O^{3'},O^{5'}$-tetrapropionylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetrabutyrylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetraisobutyrylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetravalerylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetraisovalerylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetracaproylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetraoctanoylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetralauroylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetrapalmitoylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetraoleoylcytidine;
$N^4,O^{2'},O^{3'},O^{5'}$-tetrastearoylcytidine; and
$N^4,O^{2'},O^{3'},O^{5'}$-tetralinoleylcytidine.

The $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines (I) can exhibit excellent central nervous system activating effects. For instance, it is observed that oral or intraperitoneal administration of these compounds to rabbits at a dose of 50—200 mg./kg. significantly lowers the respective intensity thresholds of such stimulation given to the mesencephalic reticular formation that evokes an arousal response in electroencephalogram and a discharge in electromyogram.

Furthermore, the $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines (I) have a low toxicity. For instance, their fifty per cent Lethal doses (LD_{50}) in rats are higher than 5000 mg./kg. when administered orally.

Thus, the $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines (I) may be used, for example, as an agent for the treatment of the disturbance of consciousness or neuro-psychiatric symptoms e.g. due to head injury, cerebral vascular disturbance or cerebral operation. The $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines (I) are administerable in the form of powders, tablets, solutions or emulsions for oral administration, or in the form of injections. The choice of the carrier is determined by the preferred route of administration, the solubility of the respective tetraacylcytidines and standard pharmaceutical practice.

Generally, the $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidines (I) are orally administered in a dose of 0.6—6 g./adult/day. A dose of 1.5—3 g./adult/day is most effective.

The following examples further illustrate the invention.

Example 1

25g. of butyric anhydride is added to a suspension of 5g. of cytidine in 100ml. of pyridine and the mixture is refluxed for 30 minutes. The reaction mixture is admixed with 30 ml. of water, left standing for about 2 hours and concentrated to dryness under a reduced pressure. The residue is dissolved in 100ml. of ethyl acetate. The solution is washed twice with 50ml. each of a 2% aqueous solution of sodium hydrogencarbonate, subsequently three times with 50ml. each of water, and is concentrated to dryness under a reduced pressure. The residue is recrystalized from 80% methyl alcohol to give 9.05g. of $N^4,O^{2'},O^{3'},O^{5'}$-tetrabutyrylcytidine as needles melting at 100°C.

Elementary analysis:

Calculated for $C_{25}H_{37}N_3O_9$:	C 57.40%,	H 7.08%,	N 8.03%
Found:	C 56.93%;	H 7.14%,	N 7.93%

Example 2

8g. of propionic anhydride is added to a suspension of 4g. of cytidine in 80ml. of pyridine and the mixture is stirred at room temperature (about 20°C) for 12 hours. The reaction mixture is admixed with 50ml. of water, left standing for about 2 hours and concentrated to dryness under a reduced pressure. The residue is dissolved in 50ml. of ethyl alcohol and the solution is concentrated to dryness under a reduced pressure, and this treatment is carried out second time.

The residue is dissolved in 10 ml. of chloroform and the solution is allowed to pass through a column packed with 100g. of silica gel. The column is subjected to elution with 1,000 ml. of chloroform to give first a fraction showing weak ultraviolet absorptions and secondly a fraction showing strong ultraviolet absorptions. The second fraction is concentrated to dryness under a reduced pressure to give 6.74g. of $N^4,O^{2'},O^{3'},O^{5'}$-tetrapropionylcytidine as a resinous material.

Elementary analysis:

Calculated for $C_{21}H_{29}N_3O_9$:	C 53.90%,	H 6.26%,	N 8.99%
Found:	C 52.77%,	H 6.39%,	N 8.43%

Example 3

16g. of caprylic anhydride is added to a suspension of 4g. of cytidine in 40ml. of pyridine and the mixture is stirred at room temperature for 12 hours.

The reaction mixture is subjected to the same isolation procedures including column chromatography employing silica gel as described in Example 2 and the resulting residue is recrystallized from 100ml. of ethyl alcohol to give 9.64g. of $N^4,O^{2'},O^{3'},O^{5'}$-tetraoctanoylcytidine as needles melting at 94°C.

Elementary analysis:

Calculated for $C_{41}H_{69}N_3O_9$:	C 65.80%,	H 9.29%,	N 5.61%
Found:	C 65.76%,	H 9.41%,	N 5.41%

Example 4

1g. of cytidine is dissolved in 200 ml. of pyridine at 50°C. 10g. of linoleyl chloride is added to the solution and the mixture is stirred at room temperature for 48 hours.

The reaction mixture is subjected to the same isolation procedures including column chromatography employing silica gel as described in Example 2 to give 4.07g. of $N^4,O^{2'},O^{3'},O^5$-tetralinoleiylcytidine as resinous material.

Elementary analysis:

Calculated for $C_{81}H_{133}N_3O_9$:	C 75.20%,	H 10.30%,	N 3.35%
Found:	C 74.93%,	H 10.37%,	N 3.64%

WHAT WE CLAIM IS:—

1. A $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidine, wherein the acyl group is derived from a fatty acid with from 3 to 18 carbon atoms.
2. A compound according to claim 1, wherein the acyl group is propionyl.
3. A compound according to claim 1, wherein the acyl group is butyryl.
4. A compound according to claim 1, wherein the acyl group is octanoyl.
5. A compound according to claim 1, wherein the acyl group is linoleyl.
6. A process for the preparation of a $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidine in which the acyl group has from 3 to 18 carbon atoms, wherein cytidine is reacted with an acid anhydride or an acid halide of the corresponding fatty acid.
7. A process according to claim 6, wherein the acid anhydride or acid halide is used in an amount in excess of 4 moles per mole of cytidine.
8. A process according to claim 7, wherein the acid anhydride or acid halide is used in an amount of from 5 to 10 moles relative to cytidine.
9. A process according to any of claims 6 to 8, wherein the reaction is carried out in an organic solvent.
10. A process according to claim 6, substantially as herein described with reference to any of the specific Examples.
11. A $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidine when prepared by a process as claimed in any of claims 6 to 10.

12. A $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidine according to claim 1 substantially as herein described with reference to any of the specific Examples.

13. A pharmaceutical composition which comprises as the active ingredient at least one $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidine wherein the acyl group has from 3 to 18 carbon atoms, together with pharmaceutically acceptable carrier or diluent therefor.

14. A pharmaceutical composition which comprises as the active ingredient at least one $N^4,O^{2'},O^{3'},O^{5'}$-tetraacylcytidine as claimed in any of claims 1 to 5, 11 and 12 together with a pharmaceutically acceptable carrier or diluent therefor.

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